

## Technical Data Sheet

## BUV395 Mouse Anti-Human CD40

## Product Information

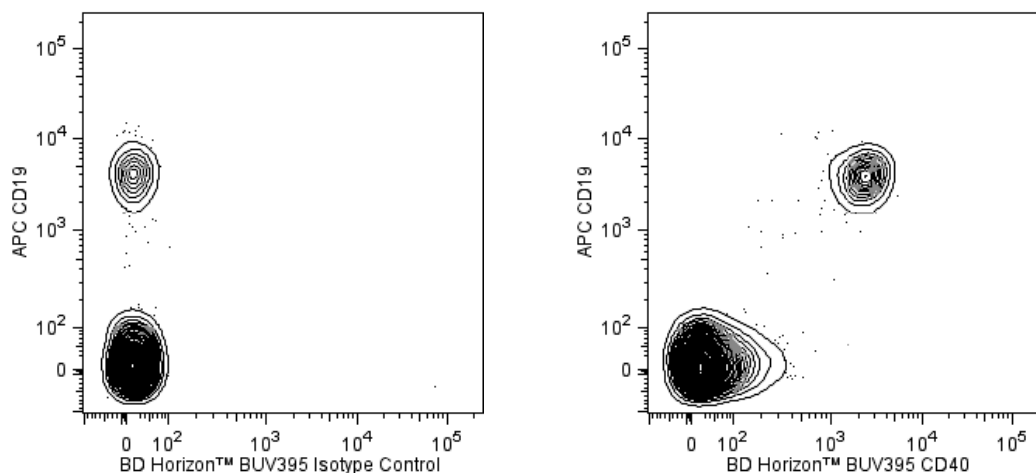
<b>Material Number:</b>	565202
<b>Alternate Name:</b>	TNFRSF5; TNF receptor superfamily member 5; CD40L receptor; Bp50; p50
<b>Size:</b>	50 Tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	5C3
<b>Isotype:</b>	Mouse IgG1, κ
<b>Reactivity:</b>	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
<b>Workshop:</b>	V CD40.4
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

## Description

This 5C3 monoclonal antibody specifically binds to CD40, a 45-48 kDa type I integral membrane glycoprotein. CD40 is expressed on B lymphocytes, but is not expressed on terminally differentiated B cells. CD40 is also expressed by endothelial cells, basal epithelial cells and some epithelial cell carcinomas, follicular dendritic cells, macrophages, fibroblasts, keratinocytes, and CD34<sup>+</sup> hematopoietic progenitor cells. This antibody is useful for studying the roles played by CD40 in B-cell growth, proliferation, and differentiation including immunoglobulin isotype switching. Anti-CD40 antibodies have been reported to stimulate B-cell proliferation when costimulated with anti-μ, anti-CD20 antibodies or with phorbol esters. 5C3 is capable of inducing B-cell proliferation when presented with IL-4.

Clone 5C3 reacts with the human form of the 45-48 kDa type I integral membrane glycoprotein, CD40. This clone also cross-reacts with a subset of peripheral blood lymphocytes, but not monocytes nor granulocytes, of baboon and both rhesus and cynomolgus macaque monkeys. The distribution on lymphocytes is similar to that seen with normal human donor lymphocytes, with the reactivity being restricted to CD20<sup>+</sup> lymphocytes.

The antibody was conjugated to BD Horizon BUV395 which has been exclusively developed by BD Biosciences as an optimal dye for use on a 355 nm laser equipped instrument. With an Ex Max at 348 nm and an Em Max at 395 nm, this dye has virtually no spillover into any other detector. BD Horizon BUV395 can be excited with a 355 nm laser and detected with a 379/28 filter.



**Two-color flow cytometric analysis of CD40 expression on human peripheral blood lymphocytes.** Whole blood was stained with APC Mouse Anti-Human CD19 antibody (555415/561742) and either BD Horizon™ BUV395 Mouse IgG1, κ Isotype Control (Cat. No. 563547; Left Panel) or BD Horizon BUV395 Mouse Anti-Human CD40 antibody (Cat. No. 565202; Right Panel). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). Flow cytometric contour plots showing the correlated expression of CD40 (or Ig isotype control staining) versus CD19 were derived from events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.

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565202 Rev. 2



## Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon BUV395 under optimum conditions, and unconjugated antibody and free BD Horizon BUV395 were removed.

## Application Notes

### Application

Flow cytometry

Routinely Tested

### Recommended Assay Procedure:

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

### Suggested Companion Products

Catalog Number	Name	Size	Clone
563794	Brilliant Stain Buffer	100 Tests	(none)
563547	BUV395 Mouse IgG1, k Isotype Control	50 µg	X40
555415	APC Mouse Anti-Human CD19	100 Tests	HIB19
561742	APC Mouse Anti-Human CD19	25 Tests	HIB19
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

### Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [wwwbdbiosciences.com/colors](http://wwwbdbiosciences.com/colors).
7. BD Horizon Brilliant Ultraviolet 395 is covered by one or more of the following US patents: 8,158,444; 8,575,303; 8,354,239.
8. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
9. Please refer to [wwwbdbiosciences.com/pharmingen/protocols](http://wwwbdbiosciences.com/pharmingen/protocols) for technical protocols.

### References

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